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A STUDY OF COMPLEMENT FIXATION IN GONORRHEAL INFECTIONS.*

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IN the year 1901 Bordet and Gengou[1] first demonstrated the method of "Complement-deviation." Afterward, in 1906, Wassermann and Bruck[2] modified Bordet's original method to what is now called "Komplementablenkungsmethode."[3] Since this method was introduced several varieties of bacteria and also protozoa have been studied by it. The study of syphilis especially by this method has been reported from every part of the world by countless numbers of authors. That it is one of the most remarkable experiments of recent times is shown by the fact that now we cannot discuss syphilis without knowing this method. The question, How does the gonococcus react with complement-binding method? is still unsettled. One year after Wassermann's publication Müller and Oppenheim[4] first studied the serum of a gonorrheal patient with this method. With serum from a patient suffering from gonorrheal arthritis, with gonococcus as antigen (NaCl emulsion of 48-hour culture on blood-agar) and with fresh guinea-pig serum as complement, then adding the beef red corpuscles and a hemolytic serum, they found that the complement was anchored by the patient's serum, hemolysis not occurring. On the contrary, the serum of a patient suffering from non-gonorrheal diseases did not anchor the complement and lysis of the erythrocytes took place. From this fact it is clear that gonorrheal serum contained a specific antibody for gonococcus. Shortly afterward Bruck[5] studied the sera of six cases of gonorrheal salpingitis, and two cases of chronic gonorrheal urethritis and obtained positive results in two of six cases of the former and in one out of the two latter. In 1907 Meakins[6] obtained two positive reactions in three cases of gonorrheal arthritis and positive results from the serum of patients suffering from pro-

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[1] *Ann. de l'Inst. Pasteur*, 1901, 15, p. 290.

[2] *Deut. med. Wchnschr.*, 1906, 32, p. 745.

[3] The term "Komplementablenkung" is frequently called in English Complement-binding, Fixation of complement, Deviation of complement, or Wassermann's reaction.

[4] *Wien. klin. Wchnschr.*, 1906, 19, p. 894.

[5] *Deut. med. Wchnschr.*, 1906, 70, p. 36.

[6] *Johns Hopkins Hosp. Bull.*, 1907, 18, p. 255.

statitis and chronic urethritis. In the same year Vannod[1] studied the serum of a rabbit immunized with the gonococcus. The antigen he used was aqueous extract of gonococcus on ascitic agar prepared by shaking for 48 hours; as complement he used guinea-pig fresh serum and added sheep erythrocytes with a specific hemolytic serum. The results showed that all immune sera anchored the complement strongly, even with the minimum doses of 0.001 c.c. of serum. In the control, normal rabbit serum never anchored the complement, the result being complete hemolysis. Serum of the rabbit immunized with meningococcus also gave complete hemolysis. The same year Wollstein[2] reported the results obtained with four cases of immune sera, using immunized rabbit serum, fresh guinea-pig serum as complement, and chicken erythrocytes with specific hemolytic serum. All immune sera gave positive reactions, no hemolysis occurring. Micrococcus catarrhalis and streptococcus as antigens gave negative results, complete hemolysis occurring; but when the antigen was meningococcus the antigonococcus serum anchored the complement and hemolysis did not occur as with gonococcic antigen; and using antimeningococcus rabbit serum with gonococcic antigen, the results also showed anchoring of the complement. Consequently one cannot differentiate between gonococcus and meningococcus by this method. Teague and Torrey[3] experimented with antigonococcic rabbit serum. The antigen was an aqueous extract of a 24-hour culture of gonococcus on Thalmann's medium. The results showed that all antigonococcal sera anchored the complement strongly, but the results were different from those of the previous workers in this, that the immune serum obtained with gonococcus A reacted strongly only with gonococcus A as antigen (homogeneous) and gave no reaction with gonococcus G or H (heterogeneous); and G- or H-serum reacted strongly only with G- or H-antigen (homogeneous) but not with A-antigen (heterogeneous). From these results they concluded that gonococci might be divided in several types as urged by Torrey[4] in previous contributions on the agglutination and precipitation of gonococcus. This is a very interesting subject for further study. I have also observed recently the same phenomenon with antigonococcic sera while working on complement-fixation. The results obtained are shown in the following experiments:

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[1] *Centralbl. f. Bakt.*, Abt. I, Orig., 1907, 44, p. 10.

[2] *Jour. Exper. Med.*, 1907, 9, p. 588.

[3] *Jour. Med. Res.*, 1907, 17, p. 223.

[4] *Ibid.*, 1907, 16, p. 329.

Human serum—
1. Normal serum; 2. Gonorrheal serum; 3. Serum with gonorrheal history; 4. Serum from patients with other diseases.
Rabbit serum—
1. Normal serum; 2. Immunized gonococcic serum; 3. Immunized meningococcic serum; 4. Torrey's gonococcic serum.
Other normal animal sera—
1. Horse serum; 2. Ox serum; 3. Goat serum.
Relation to agglutination.
Conclusions.

Method.—For the experiments five different agents are required; the procedure is as follows:

Amboceptor: 24–48-hour culture of gonococcus on blood-agar (horse blood) emulsified in normal salt solution, and this living emulsion placed in a shaking apparatus for 48 hours, then injected intraperitoneally into a rabbit at intervals of a week to 10 days with increasing doses. About 12–15 days after last injection (8 to 10 times) the rabbit is bled and tested for the experiments.

Antigen: a slant culture of 24–48 hours on blood-agar emulsified in 5 c.c. of sterile distilled water and heated at 60° C. for ½ hour, then placed in a shaking apparatus for 48 hours and centrifugalized. As a result of this preparation the fluid was not, however, completely cleared. The extract was kept in the ice-room and it was never used when more than three days old (just five days after culture). For controls B. coli, meningococcus, and Staph. aureus emulsion were made under the same conditions. For the experiments the gonococcus as antigen had been isolated from the same sources as given in a previous paper[1] by me.

Complement: Normal guinea-pig freshly bled and the serum placed in the ice-room for 16 to 24 hours. Rabbit serum as complement is frequently very variable in power, guinea-pig serum as complement has almost the same power, and I never failed to get complete hemolysis with it. Rabbit complement sometimes showed no hemolysis, unless it was used in large doses, when it caused complete hemolysis, showing that rabbit's serum used as complement is inconstant. My dose of guinea-pig's complement is 0.05 c.c.

Hemolytic sera: Freshly defibrinated goat's blood corpuscles injected intraperitoneally into a healthy rabbit at intervals with increasing doses as 2, 5, 10, and 20 c.c. About 10 days after last injection the serum was tested by experiments as to its hemolytic power for a solution of 5 per cent erythrocytes, from undiluted serum to 0.001–0.0025 c.c. To the hemolytic serum 0.5 per cent carbolic acid was finally added and the whole kept in the ice-room until required for use.

Erythrocytes: Goat's blood corpuscles from freshly defribinated blood, the washed erythrocytes made into a 5 per cent suspension by adding 0.9 per cent salt solution.

Small test tubes were used for experiments. First the antigen (0.1 c.c.), amboceptor (certain doses), and complement (0.05 c.c.) were well mixt in the tubes and placed in incubator at 37° C. for one hour. During this period the tubes were shaken 3 to 4 times. Next the hemolytic sera and erythrocytes were added and the tubes placed in the incubator at 37° C. for 2 hours, shaken 4 or 5 times at intervals. I used certain strengths of hemolytic serum added to 5 per cent erythrocytes; for instance, if the hemolytic title was 0.001 c.c. for 1 c.c. of 5 per cent suspension, 0.1 c.c. of this hemolytic serum was added directly to 100.0 c.c. of 5 per cent suspension, and 1 c.c. of this mixture added to the previous agents. After 2 hours in the incubator, the tubes were kept in ice-room for 24 hours and the results observed. The following table shows details of the technic.

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[1] *Jour. Med. Res.*, 1909, 20, p. 365.

TABLE 1.

	Antigen	Amboceptor			Complement 2X	0.9% NaCl Sol.		5% Erythrocytes with Certain Hemolytic Sera		Results
1	0.1	0.1	10X	1.0	0.05 (0.1)	0	37° C. for 1 hour	1.0	37° C. for 2 hours	after 24 hours in ice-room
2	"	0.05		0.5	"	0.5		"		
3	"	0.025		0.25	"	0.75		"		
4	"	0.01	100X	1.0	"	0		"		
5	"	0.005		0.5	"	0.5		"		
6	"	0.0025		0.25	"	0.75		"		
7	"	0.001	1000X	1.0	"	0		"		
Control I	0	0			0.05	1.0		"		
Control II	0	0.1			0	0		"		
Control III	0.1	0			0.05	1.0		"		
Control IV	0	0.1			0.05	0		"		
Control V	0	0			0	0		"		

NOTE.—Results were indicated as follows: – = no hemolysis, ± = trace, + = hemolysis, ++ = strong hemolysis, +++ = complete hemolysis, ? = not tested.

HUMAN SERUM.

Normal serum.—Before experimenting with gonorrheal serum I examined normal human serum for its reaction. Healthy human serum, with absolutely no history of any gonorrheal infection, was taken from a blister and inactivated at 55° C. for one hour, then tested, the results being as follows:

TABLE 2.

O. K., aged 25, never affected by gonorrhea, at present in healthy condition. His serum did not fix complement.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	+++	+++	+++	+++	–	–	+++	+++	–	+++
0.05	+++	+++	+++	+++	±	±	+++	+++	+	+++
0.025	+++	+++	+++	+++	+	+	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control II	–	–	–	–	–	–	–	–	–	–
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control IV	+++	+++	+++	+++	–	–	+++	+++	±	+++
Control V	–	–	–	–	–	–	–	–	–	–

As the table shows, complete hemolysis occurred with the gonococcus strains A, B, C, D, G, H, and B. coli, as no complement was fixt, but with gonococcus E and F, and meningococcus no hemolysis occurred in doses of 0.1 c.c. This may be supposed however to be a normal condition like the normal agglutination property of the serum, because 0.1 c.c. of serum is a comparatively large amount. Therefore I think this is a normal condition, not a specific reaction.

TABLE 3.

S. A., aged 27, never affected by gonorrhea, at present suffering from dysentery (bacillary). Serum taken three weeks after the onset of the dysentery.

SERUM		GONOCOCCUS STRAINS								MEN-INGO-COC-CUS	B. COLI
		A	B	C	D	E	F	G	H		
0.1		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.05		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.025		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control	I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	II	–	–	–	–	–	–	–	–	–	–
	III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	IV	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	V	–	–	–	–	–	–	–	–	–	–

This serum did not fix the complement for any of the antigens.

Following sera were taken from patients who may or may not have had gonorrhea previously, but at the time were healthy. The answers about a gonorrheal history could not be relied upon; therefore, it is only so-called healthy serum, but whether affected previously or not by gonorrhea could not be known for certain.

TABLE 4.

T. S., aged 30, at present in a healthy condition, denied having ever been affected by gonorrhea.

SERUM		GONOCOCCUS STRAINS								MEN-INGO-COC-CUS	B. COLI
		A	B	C	D	H	F	G	H		
0.1		–	–	–	–	–	–	–	–	+	++
0.05		–	–	–	–	–	–	–	–	++	+++
0.025		–	–	–	+	–	++	±	–	+++	+++
0.01		–	+	±	+	–	++	++	+	+++	+++
0.005		+	++	+++	+++	±	+++	++	++	+++	+++
0.0025		++	+++	+++	+++	++	+++	+++	+++	+++	+++
0.001		+++	+++	+++	+++	++	+++	+++	+++	+++	+++
Control	I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	II	–	–	–	–	–	–	–	–	–	–
	III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	IV	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	V	–	–	–	–	–	–	–	–	–	–

This serum caused no hemolysis because the complement was fixt, showing that T. S. serum might have contained gonococcic amboceptor. I again asked him for a gonorrheal history, but the answer was the same as before, that he never had gonorrhea. Then I asked his friend secretly, who was living in the same room with him. The friend said that T. S. had been suffering from gonorrheal urethritis for the past four weeks. The friend's answer explained clearly the positive reaction as shown by result.

TABLE 5.

S. T., aged 18, present condition healthy, said he never had gonorrheal infection.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	++	+++	−	+++	+	?	++	−	++	+++
0.05	+++	+++	++	+++	++	?	+++	+	+++	+++
0.025	+++	+++	+++	+++	+++	?	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	?	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	?	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	?	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	?	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	?	+++	+++	+++	+++
II	−	−	−	−	−	?	−	−	−	−
III	+++	+++	+++	+++	+++	?	+++	+++	+++	+++
IV	+++	+++	−	+++	+++	?	+++	−	+++	+++
V	−	−	−	−	−	?	−	−	−	−

The result shows that the serum had no gonococcic amboceptor. The subject may not have had gonorrheal infection. For this experiment the antigen of gonococcus F was not prepared because, in shaking, the tube was broken by accident.

Serum of gonorrheal patients.

TABLE 6.

R. W., aged 39, first attacked by gonorrhea 20 years ago; since that time he has had a recurrence of the same infection five times. At present his physician diagnosed him as suffering from chronic gonorrheal urethritis.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	++	+++	++	++	+++	+	+	+++	+++	+++
0.05	+++	+++	+++	+++	+++	+++	+	+++	+++	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	−	−	−	−	−	−	−	−	−	−
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+++	+++	+++	+++	+++	+	+	+++	+++	+++
V	−	−	−	−	−	−	−	−	−	−

The table shows that the serum has no amboceptor to any other gonococcus strains, than gonococcus E which was isolated from this patient. This homogeneous extract showed complete hemolysis (fixt complement).

TABLE 7.

Y. M., aged 30, diagnosed as chronic gonorrheal urethritis and cystitis.

SERUM	GONOCOCCUS STRAINS								MEN-INGO-COC-CUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	–	–	–	–	–	–	–	–	–	++
0.05	–	–	–	–	+	–	–	–	+	+++
0.025	+	–	±	–	++	–	+	–	++	+++
0.01	+++	++	++	–	+++	–	++	+++	+++	+++
0.005	+++	+++	+++	+	+++	+	+++	+++	+++	+++
0.0025	+++	+++	+++	++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control II	–	–	–	–	–	–	–	–	–	–
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control IV	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control V	–	–	–	–	–	–	–	–	–	–

Gonococcus F was isolated from this patient. All strains show positive reactions, especially F (homogeneous) and D which reacted in the same manner very strongly. From this it may be assumed that strains F and D belong to the same group.

TABLE 8.

C. H., aged 25, attacked by gonorrheal infection three years ago; since that time the disease has persisted as chronic gonorrheal urethritis, cystitis, and epididymitis.

SERUM	GONOCOCCUS STRAINS								MEN-INGO-COC-CUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	–	–	–	–	+	–	–	–	–	?
0.05	–	–	–	–	+	–	–	–	+	?
0.025	–	–	–	+	+	±	–	–	+++	?
0.01	–	–	–	+	++	++	+	–	+++	?
0.005	–	–	+	++	+++	+++	+++	–	+++	?
0.0025	–	–	++	+++	+++	+++	+++	–	+++	?
0.001	++	+	+++	+++	+++	+++	+++	+	+++	?
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	?
Control II	–	–	–	–	–	–	–	–	–	?
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++	?
Control IV	+++	+++	+++	+++	+++	+++	+++	+++	+++	?
Control V	–	–	–	–	–	–	–	–	–	?

Gonococcus H was isolated from this patient. Reaction was positive for A, B, C, D, E, F, G, and H (homogeneous). Gonococcus A and B reacted in equal strength to the serum, showing H, A, and B to be of the same group.

TABLE 9.

K. U., aged 35, diagnosed as secondary stage of syphilis and chronic gonorrheal endometritis.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	−	−	−	−	−	−	−	−	+	++
0.05	−	−	−	−	−	−	−	−	+	++
0.025	−	−	−	+	−	±	−	−	+++	+++
0.01	−	−	+	++	−	+	−	−	+++	+++
0.005	−	−	+++	+++	−	+++	±	−	+++	+++
0.0025	+	−	+++	+++	++	+++	++	±	+++	+++
0.001	++	+++	+++	+++	+++	+++	+++	++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	−	−	−	−	−	−	−	−	−	−
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
V	−	−	−	−	−	−	−	−	−	−

The gonococcus could not be isolated from this patient, so this serum could not be tested with homogeneous strain as antigen. However, all strains showed positive reactions, especially A, B, C, E, G, and H, which reacted somewhat similarly; therefore it may be assumed that if a gonococcus was isolated from this patient, that gonococcus might have reacted the same as strains A, B, C, E, G, and H and belong to the same group (see Table 19, gncc. imm. serum).

TABLE 10.

T. K., aged 22, acute gonorrheal urethritis, two weeks after infection.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	+++	±	+++	++	+++	+++	+	+	+++	+++
0.01	+++	++	+++	++	+++	+++	+++	++	+++	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	−	−	−	−	−	−	−	−	−	−
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+++	−	+++	+++	+++	+++	+	+	+++	+++
V	−	−	−	−	−	−	−	−	−	−

Gonococcus A was isolated from this patient, but the homogeneous extract as the antigen and other heterogeneous antigens did not fix the complement; complete hemolysis occurred. Gonococcus B showed slight hemolysis in 0.1 c.c. but this is a normal reaction as previously mentioned.

TABLE 11.

H. S., aged 20, acute gonorrheal urethritis, five days after infection.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	+++	+++	+++	+++	+++	+	+++	+++	+++	+++
0.05	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	−	−	−	−	−	−	−	−	−	−
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+++	+++	+++	+++	+++	+	+++	+++	+++	+++
V	−	−	−	−	−	−	−	−	−	−

Gonococcus C was isolated from this patient, but the results were all negative.

Concerning the last two tables we note that acute gonorrheal sera do not fix the complement to the antigens.

Serum from patients with a history of gonorrhea but no disease at present.

TABLE 12.

K. K., aged 30, first infection five years ago. Affected several times since, but present condition healthy.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	−	±	−	−	−	−	−	+++	++	±
0.05	−	+++	±	−	+	−	−	+++	+++	+++
0.025	+++	+++	+++	++	++	+++	−	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	−	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	−	−	−	−	−	−	−	−	−	−
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	±	±	++	+	−	+++	+++	+++	+++	+
V	−	−	−	−	−	−	−	−	−	−

The results show that gonococcus G fixt the complement to this serum. It may be supposed that if the gonococcus had been isolated from this patient it might be of the same group as the G strain.

TABLE 13.

H. T., aged 25, affected by acute gonorrheal urethritis three years before; since that time he never has been affected again and at present in a healthy condition.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	+++	+++	+++	+++	+	+++	+++	++	++	+
0.05	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	–	–	–	–	–	–	–	–	–	–
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
V	–	–	–	–	–	–	–	–	–	–

This shows entirely negative results.

Serum from patients with other diseases.

TABLE 14.

S. S., aged 25, serum taken 10 days after diagnosis of typhoid fever. No history of any gonorrheal infection.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	+++	+++	?	+++	+++	+++	+++	+++	+++	+++
0.05	+++	+++	?	+++	+++	+++	+++	+++	+++	+++
0.025	+++	+++	?	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	?	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	?	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	?	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	?	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	?	+++	+++	+++	+++	+++	+++	+++
II	–	–	?	–	–	–	–	–	–	–
III	+++	+++	?	+++	+++	+++	+++	+++	+++	+++
IV	+++	+++	?	+++	+++	+++	+++	+++	+++	+++
V	–	–	?	–	–	–	–	–	–	–

The result was negative.

TABLE 15.

S. R., aged 24, diagnosed as catarrhal pleurisy; gonorrheal history is unknown; the pleural exudate was examined.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	+++	+++	+++	++	+	+++	+++	+++	+++	+++
0.05	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control II	–	–	–	–	–	–	–	–	–	–
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control IV	+++	+++	+++	+++	+	+++	+++	+++	+++	+++
Control V	–	–	–	–	–	–	–	–	–	–

The result was negative.

RABBIT SERUM.

Normal rabbit serum.

TABLE 16.

Rabbit I, weight 2,400 gm., healthy.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	–	–	+++	+++	+	+++	–	+++	±	±
0.05	±	±	+++	+++	+++	+++	±	+++	+++	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control II	–	–	–	–	–	–	–	–	–	–
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control IV	–	±	++	+++	+	+++	–	+++	+	±
Control V	–	–	–	–	–	–	–	–	–	–

Results are all negative. Some of the experiments, however, showed no hemolysis with 0.1 c.c. of the serum. This may be the limit to normal reaction, not specific.

TABLE 17.

Rabbit III, 2,500 gm., healthy.

Serum	Gonococcus Strains								Meningococcus	B. coli
	A	B	C	D	E	F	G	H		
0.1	−	+++	−	+++	+	−	+++	+++	+++	+++
0.05	±	+++	±	+++	+++	+	+++	+++	+++	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control II	−	−	−	−	−	−	−	−	−	−
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control IV	−	+++	±	+++	+	−	+++	+++	+++	+++
Control V	−	−	−	−	−	−	−	−	−	−

The results are similar to normal serum of Rabbit I.

TABLE 18.

Rabbit V, 1,600 gm., healthy, normal.

Serum	Gonococcus Strains								Meningococcus	B. coli
	A	B	C	D	E	F	G	H		
0.1	±	+	±	+++	+	+++	+++	+++	++	+++
0.05	+++	+++	+++	+++	++	+++	+++	+++	+++	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control II	−	−	−	−	−	−	−	−	−	−
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control IV	±	+	±	+++	+	+++	+++	+++	+++	+++
Control V	−	−	−	−	−	−	−	−	−	−

As seen from the three tables above the results are all similar in reaction, and it may be accepted that normal rabbit sera do not fix the complement to gonoccocic antigen, no hemolysis occurring except in 0.1 to 0.05 c.c. which is a normal condition like that of human serum.

Immunized rabbit serum.—Rabbits were highly immunized with eight different strains of gonococcus, consisting of 24- to 48-hour growths from blood-agar (original test tube), emulsified in normal salt solution, and heated at 55° C. for one hour, then injected intravenously with intervals of five days. After third injection, living gonococcic emulsion was injected intraperitoneally at intervals of a week with increasing doses. Altho various strains of gonococcus differ greatly in toxicity, the animals seldom succumb to this treatment. After the injection the animals lost weight markedly and some died after third or fourth inoculation or even a few days after first injection. It is clear that immunization of animals with gonococcus is not easy, however; usually after the fifth inoculation the animals became thoroughly immunized and began to gain weight. After the rabbit had received 10 inoculations extending over a period of about two months the blood was drawn from the carotid 10 to 15 days after last injection, and the various immunized rabbit sera used for the experiments. The results are as follows:

TABLE 19.

RABBIT IMMUNIZED TO GONOCOCCUS A.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	−	−	−	−	−	−	−	−	−	+++
0.05	−	−	−	−	−	−	−	−	+	+++
0.025	−	−	−	−	−	−	−	−	++	+++
0.01	−	−	−	+	−	+	−	−	+++	+++
0.005	−	−	−	++	±	++	−	−	+++	+++
0.0025	−	−	±	+++	±	+++	−	+	+++	+++
0.001	+	±	++	+++	+	+++	+	++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	−	−	−	−	−	−	−	−	−	−
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+++	+++	+++	+++	+	+++	++	+++	±	+++
V	−	−	−	−	−	−	−	−	−	−

As seen from the above results immunized gonococcic serum A reacted strongly to gonococcus A (homogeneous), B, C, E, G, and H. This interested me very much, as by comparing the positive reaction of the gonorrheal patients' sera (see Table 9) to gonococcus strains A, B, and H, it seems to me this shows clearly gonococcus strains A, B, C, E, G, and H belong to the same group.

TABLE 20.

RABBIT IMMUNIZED TO GONOCOCCUS B.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	−	−	−	−	−	−	−	−	±	+++
0.05	−	−	−	−	−	−	−	−	+++	+++
0.025	−	−	−	−	−	−	−	−	+++	+++
0.01	−	−	−	+	−	±	−	−	+++	+++
0.005	−	−	−	++	−	+	−	−	+++	+++
0.0025	−	−	−	+++	+	++	−	−	+++	+++
0.001	−	−	−	+++	+	++	±	−	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	−	−	−	−	−	−	−	−	−	−
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
V	−	−	−	−	−	−	−	−	−	−

The results show that strains A, B, C, E, G, and H are of the same group.

TABLE 21.

RABBIT IMMUNIZED TO GONOCOCCUS C.

SERUM		GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
		A	B	C	D	E	F	G	H		
0.1		–	–	–	–	–	–	–	–	+	+
0.05		–	–	–	–	–	–	–	–	+++	++
0.025		–	–	–	±	–	±	–	–	+++	+++
0.01		–	–	–	++	–	+	–	–	+++	+++
0.005		–	–	–	+++	–	++	–	–	+++	+++
0.0025		±	+	–	+++	±	+++	–	–	+++	+++
0.001		+	+	±	+++	++	+++	+	–	+++	+++
Control	I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	II	–	–	–	–	–	–	–	–	–	–
	III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	IV	+	+++	–	+++	++	+++	++	+	+	+
	V	–	–	–	–	–	–	–	–	–	–

As seen from the above strains C, and A, B, E, G, H, belong to the same group.

TABLE 22.

RABBIT IMMUNIZED TO GONOCOCCUS D.

SERUM		GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
		A	B	C	D	E	F	G	H		
0.1		–	–	–	–	–	–	–	–	–	±
0.05		–	–	–	–	–	–	–	–	±	+++
0.025		–	+	+	–	+	–	+	+++	+++	+++
0.01		+++	+++	+++	–	+	–	+	+++	+++	+++
0.005		+++	+++	+++	–	+	–	+	+++	+++	+++
0.0025		+++	+++	+++	–	+	±	+++	+++	+++	+++
0.001		+++	+++	+++	–	+	+	+++	+++	+++	+++
Control	I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	II	–	–	–	–	–	–	–	–	–	–
	III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	IV	–	++	+++	+	+++	+++	+++	+++	+	±
	V	–	–	–	–	–	–	–	–	–	–

The results show that strains D and F give the same reaction, that is, they belong to the same group. The previous table also shows D and F had the same reaction from gonorrheal patients' sera. There is a decided difference between the D and F, and the A, B, C, E, G, and H strains.

TABLE 23.
RABBIT IMMUNIZED TO GONOCOCCUS E.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	–	–	–	–	–	–	–	–	–	–
0.05	–	–	–	–	–	–	–	–	++	++
0.025	–	–	–	–	–	+	–	–	+++	+++
0.01	–	–	–	++	–	++	–	–	+++	+++
0.005	–	–	–	+++	–	+++	–	–	+++	+++
0.0025	–	–	–	+++	±	+++	–	–	+++	+++
0.001	+	±	±	+++	+	+++	–	–	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	–	–	–	–	–	–	–	–	–	–
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+	+	–	–	++	+	–	+++	±	–
V	–	–	–	–	–	–	–	–	–	–

The results show that gonococcus E serum reacted to A, B, C, G, and H strains, i. e., they all belong to the same group.

TABLE 24.
RABBIT IMMUNIZED TO GONOCOCCUS F.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	–	–	–	–	–	–	–	–	+++	+++
0.05	–	–	–	–	–	–	–	–	+++	+++
0.025	–	–	±	–	–	–	–	–	+++	+++
0.01	±	±	+	–	+++	–	+	+	+++	+++
0.005	+++	++	++	–	+++	–	+++	+	+++	+++
0.0025	+++	+++	+++	–	+++	–	+++	+	+++	+++
0.001	+++	+++	+++	+	+++	–	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	–	–	–	–	–	–	–	–	–	–
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
V	–	–	–	–	–	–	–	–	–	–

This shows that strains D and F clearly belong to the same group.

TABLE 25.
RABBIT IMMUNIZED TO GONOCOCCUS G.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	–	–	–	–	–	–	–	–	++	+++
0.05	–	–	–	–	–	–	–	–	+++	+++
0.025	–	–	–	–	–	–	–	–	+++	+++
0.01	–	–	–	++	–	±	–	–	+++	+++
0.005	–	–	–	++	–	+++	–	–	+++	+++
0.0025	–	+	+	+++	–	+++	–	–	+++	+++
0.001	–	+	+	+++	–	+++	–	±	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	–	–	–	–	–	–	–	–	–	–
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+++	+++	+	++	+++	+++	++	+++	++	+++
V	–	–	–	–	–	–	–	–	–	–

This shows, strain G and strains A, B, C, E, H, are of the same group.

TABLE 26.

RABBIT IMMUNIZED TO GONOCOCCUS H.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	–	–	–	–	–	–	–	–	+++	+++
0.05	–	–	–	–	–	–	–	–	+++	+++
0.025	–	–	–	–	–	–	–	–	+++	+++
0.01	–	–	–	++	–	+	–	–	+++	+++
0.005	–	–	–	+++	–	+	–	–	+++	+++
0.0025	–	+	–	+++	+	+++	–	–	+++	+++
0.001	–	+	±	+++	+	+++	–	–	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	–	–	–	–	–	–	–	–	–	–
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
V	–	–	–	–	–	–	–	–	–	–

This shows again that H and A, B, C, E, and G, belong to the same group.

Meningococcus immune rabbit serum.

TABLE 27.

RABBIT IMMUNIZED TO MENINGOCOCCUS.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	+	+++	–	+++	–	–	+++	+++	–	+++
0.05	+++	+++	+++	+++	+	–	+++	+++	–	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	–	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	–	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	–	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	–	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	–	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	–	–	–	–	–	–	–	–	–	–
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+	+++	±	+++	–	–	+++	+++	++-	+++
V	–	–	–	–	–	–	–	–	–	–

The results show that all gonococcus strains have not reacted positively. Normal reaction, however, was limited to the serum of 0.1 to 0.05 c.c. dilutions. On the other hand meningococcic antigen reacted positively with strong fixation of complement, showing that a certain differentiation between meningococcus and gonococcus may be determined by this method. At present time does not permit me to test this reaction upon several strains of meningococcus; it must be reserved for future observations.

TORREY'S ANTIGONOCOCCIC SERUM.

The Tokio branch office of Parke Davis & Co. supplied me with the sample of Torrey's antigonococcic serum. It may be added that Torrey's serum is prepared by immunizing a goat with several stains of gonococcus. The serum which I used was two months old, counting from the time it was sent out from the Parke Davis laboratory. I have tested this comparatively old Torrey's serum upon the complement-binding reaction, the results being as follows:

TABLE 28.

TORREY'S ANTIGONOCOCCIC SERUM.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	−	−	−	−	−	−	−	−	−	+
0.05	−	−	−	−	−	+	−	−	++	+++
0.025	−	−	−	±	−	+	−	−	+++	+++
0.01	+	−	−	+	−	+	−	−	+++	+++
0.005	++	+	+++	+	+	+++	+	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
II	−	−	−	−	−	−	−	−	−	−
III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV	+++	+++	±	+++	+++	+++	++	±	−	+
V	−	−	−	−	−	−	−	−	−	−

As seen from the above table the results show the same reactions to all gonococcus strains. This preparation of course is old and contained probably several different strains of gonococcus and it may be has lost its original power of "complement-binding." If the serum was new it might react positively and strongly. In any case I could not find any different strains or what groups were contained in this sample of Torrey's antigonococcic serum from my eight strains of gonococcus.

OTHER NORMAL ANIMAL SERA.

I have examined the sera of the horse, ox, and goat; the results are as follows:

TABLE 29.
NORMAL HORSE SERUM I.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	+++	+++	+++	±	+++	+++	+++	+++	+++	+++
0.05	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control II	–	–	–	–	–	–	–	–	–	–
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control IV	+++	+++	+++	++	+++	+++	+++	+++	+++	+++
Control V	–	–	–	–	–	–	–	–	–	–

TABLE 30.
NORMAL HORSE SERUM II.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS
	A	B	C	D	E	F	G	H	
0.1	–*	–*	±	+*	+	–*	–*	±	+
0.05	++	+++	+++	+++	+++	++	+++	++	++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control II	–	–	–	–	–	–	–	–	–
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control IV	–*	–*	+	++	++	–*	–*	±	+++
Control V	–	–	–	–	–	–	–	–	–

* Normal phenomenon: this is more frequent in horse serum than other serum.

As seen from the tables normal horse serum never fixes the complement to gonococcic antigen.

TABLE 31.
NORMAL OX SERUM I.

SERUM	GONOCOCCUS STRAIN								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.05	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control II	–	–	–	–	–	–	–	–	–	–
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control IV	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control V	–	–	–	–	–	–	–	–	–	–

TABLE 32.

NORMAL OX SERUM II.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.05	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control II	−	−	−	−	−	−	−	−	−	−
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control IV	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control V	−	−	−	−	−	−	−	−	−	−

Normal ox serum does not fix the complement to any gonococcic antigens.

TABLE 33.

NORMAL GOAT SERUM.

SERUM	GONOCOCCUS STRAINS								MENINGOCOCCUS	B. COLI
	A	B	C	D	E	F	G	H		
0.1	+	+++	+	+	+++	+	+++	+	+++	?
0.05	+	+++	+	+++	+++	+++	+++	+++	+++	?
0.025	+++	+++	+++	+++	+++	+++	+++	+++	+++	?
0.01	+++	+++	+++	+++	+++	+++	+++	+++	+++	?
0.005	+++	+++	+++	+++	+++	+++	+++	+++	+++	?
0.0025	+++	+++	+++	+++	+++	+++	+++	+++	+++	?
0.001	+++	+++	+++	+++	+++	+++	+++	+++	+++	?
Control I	+++	+++	+++	+++	+++	+++	+++	+++	+++	?
Control II	−	−	−	−	−	−	−	−	−	?
Control III	+++	+++	+++	+++	+++	+++	+++	+++	+++	?
Control IV	+	+++	+	+	+++	+	+++	++	+++	?
Control V	−	−	−	−	−	−	−	−	−	?

As seen from the above tables sera of normal animals never react positively for any strains of gonococcus.

RELATION TO AGGLUTINATION.

My observation of agglutination, with the same strains of gonococcus as tested with the complement-binding reaction, showed that there is no relation between complement-binding reaction and agglutination. This fact agrees with Bruck's studies. The details of the experiments will be published in the near future.

CONCLUSIONS.

The study of different species of bacteria is an important problem at the present time. Several strains of B. dysenteriae and B. coli and others have been described and are differentiated by their reaction on carbohydrate media, but observations of the gonococcus upon carbohydrate media have not yet shown any different strains. Torrey and Teague have studied the gonococcus in regard to the agglutination, precipitation, and complement-binding reactions and have shown that the strains may be divided into several types. According to their experiments from ten strains three different groups were isolated. Can the gonococcus be proved to have several species by the serum reaction? I have studied this question by the complement-binding method, the results being as above described. Concerning my experiments, eight strains of gonococcus may be separated into two different groups. There is not, however, a distinct difference between them like that of different types of B. dysenteriae as shown on carbohydrate media. It seems that the gonococcus has not many types, but at present our knowledge of the different types of bacteria is still imperfect. I failed to isolate many different groups and my experiments, unlike the results of Torrey, have not shown any distinct differentiation among them. His experiments have shown only positive reactions to homogeneous, but never positive to heterogeneous strains; it is, therefore, difficult to differentiate between various types of gonococcus and other bacteria by this method. But my experiments have shown no distinct difference among the various strains of gonococcus, only a comparative difference, and it is very easy to differentiate between any gonococcus and other bacteria.

The following general conclusions may be drawn from the experiments I have described:

1. Normal human serum has frequently marked hemolysis in 0.05 to 0.1 c.c. of the serum. This is not a specific reaction, but seems to be a normal reaction like normal agglutination (group reaction). In normal serum, however, hemolysis never occurred in dilutions below 0.05 c.c. of the serum.

2. The sera of gonorrheal patients contain specific amboceptors for the complement-binding reaction. In chronic cases the results are usually positive. Of six cases on which I carried out my experi-

ments three chronic cases showed positive reactions and one chronic and two acute cases gave negative reactions.

3. In serum of previously gonorrheal patients, well at the time of the test, one chronic case was positive and one acute case negative.

4. The sera of patients suffering from other diseases, viz., typhoid fever and catarrhal pleurisy, showed negative reaction.

5. Of the three experiments carried out with normal rabbit serum no complement was fixt for any strains of gonococcus. In doses of 0.1 c.c. of the serum, however, hemolysis frequently marked the reaction like the normal reaction of normal human serum.

6. Of eight strains of immunized rabbit serum six gave a strong reaction for six certain gonococcus strains which may belong to the same group, and two sera gave strong reaction for two certain gonococcus strains which may be classified as another group; so this divides the eight strains into two different groups. But this difference is only comparative.

7. As the control antimeningococcic serum gave a positive reaction for meningococcic antigen only, and a negative for gonococcic antigen, this reaction will differentiate between these species. Also the complement-binding reaction upon the different species of gonococcus was stronger than the reaction with meningococcus used as control. It is not, therefore, difficult to differentiate meningococcus from gonococcus by this method.

8. As controls normal sera of other animals, two horses, two oxen, and one goat, were tested. The sera all gave negative reactions and the so-called normal reaction did not occur as with normal human and rabbit sera.

9. There is no relation whatever between the complement-binding reaction and the agglutination reaction.